Amendments to the Specification:

The following is a marked-up version the Specification pursuant to revised 37 C.F.R. §1.121, with instructions and markings showing changes made herein to the Specification as filed. Underlining denotes added text, while strikeout denotes deleted text.

On page 1, after the title, please amend the first paragraph as indicated:

The present application is a Continuation application of currently pending U.S. Patent Application Serial Number 10/037,677, filed October 23, 2001, now, U.S. Patent No. 6,706,503, which is a Divisional of U.S. Patent Application Serial Number 09/314,847, filed May 19, 1999, now U.S. Patent No. 6,365,410.

Please replace the paragraph beginning at page 9, line 33, with the following rewritten paragraph:

Methods of the present invention are especially advantageous for producing improved microorganisms used for the biocatalytic production of chemicals and vitamins where numerous catalytic events are taking place either concurrently or sequentially within the host microorganism. In such complex biocatalytic systems, it is often difficult to identify the specific molecular events causing low yields, host toxicity or catalytic failures and therefore difficult if not impossible to understand which specific genetic events to alter in order to correct the deficiencies. The methods of the present invention provide the advantage of allowing the microorganism to make the required changes in response to selective pressure. Indeed, the present invention provides methods for producing a protein from an evolved microorganism comprising the steps of: a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein; b) culturing the microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein the heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type, and wherein the heterologous protein is expressed by the microorganism; and c) restoring the evolved microorganism to a wild type mutation rate. In some preferred embodiments, the methods further comprise the step of isolating at least one heterologous protein from said evolved microorganism. In some particularly preferred embodiments, the at least one heterologous protein is a hydrolase. In yet

further preferred embodiments, the hydrolase is selected from the group consisting of proteases, esterases, lipases, phenol oxidase, permeases, amylases, pullulananses, cellulases, glucose isomerase, laccases, and protein disulfide isomerases.

Please add the following paragraphs after the above-amended paragraph (i.e., page 10, after line 6, of the present application.

The present invention further provides methods for producing a heterologous protein in an evolved microorganism comprising the steps of: a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein at least one heterologous protein is an enzyme necessary for an enzymatic pathway; b) culturing the microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein the heterologous mutator gene generates a mutation rate of at least 5 to100,000-fold relative to wild type; and c) restoring the evolved microorganism to a wild type mutation rate. In some embodiments, the enzyme is selected from the group consisting of reductases and dehydrogenases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of ascorbic acid or ascorbic acid intermediates. In some alternative embodiments, the enzyme is selected from the group consisting of glycerol dehydratase and 1,3-propanediol dehydrogenase, and further wherein the enzymatic pathway results in the production of at least one compound selected from the group consisting of 1,3-propanediol, 1,3-propanediol precursors, and 1,3-propanediol derivatives. In some embodiments, the enzyme is selected from the group consisting of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatases, and further wherein the enzymatic pathway results in the production of at least one compound selected from the group consisting of glycerol and glycerol derivatives. In additional embodiments, the evolved microorganism expresses at least one heterologous protein. In still further embodiments, the methods of the present invention further comprise the step of isolating the at least one heterologous protein from the evolved microorganism. In some particularly preferred embodiments, the microorganism is selected from the group consisting of *E. coli* and *E. blattae*. In yet additional embodiments, the microorganism comprises a plasmid comprising the heterologous mutator gene and the step of restoring the evolved microorganism to a wild type mutation rate comprises curing the evolved

microorganism of the plasmid. In some embodiments, the plasmid comprises a temperature sensitive origin of replication. In yet further embodiments, the mutator gene comprises at least one *mutD* mutation. In alternative embodiments, the mutator gene comprises at least one mutator gene selected from the group consisting of comprises *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, and *mutU* mutations or homologues thereof.